IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	
MORISHITA ET AL.	:	GROUP ART UNIT: 1636
Serial No. 09/029,497	:	
Filed: June 9, 1998	:	EXAMINER: R.Schwartzman
For: MEDICAMENT CONTAINING HGF GENE	:	

D E C L A R A T I O N

Honorable Commissioner of
Patents & Trademarks
Washington, D. C. 20231

Sir:

I, Ryuichi MORISHITA, a Japanese citizen, c/o 11-22-502, Miyahara 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532 Japan, declare and state as follows:

1. I am the first inventor of the above-identified application.

2. An up to date copy of my curriculum vitae is attached hereto. As can be seen from the curriculum vitae, I currently hold the positions of Chief of the Section of Gene Therapy, Department of Geriatric

Medicine, Osaka University Medical School, and Chief of the Section of Cardiovascular Medicine, Division of Gene Therapy Science, Osaka University Medical School. I obtained the qualification of MD from Osaka University Medical School in Japan in 1987, and the qualification of PhD from Osaka University Medical School in Japan in 1991. I am an author of 145 scientific publications. My research interests include a gene therapy using a HGF gene.

3. I am familiar with the prosecution history of the above-identified application. I have read the Final Official Action dated August 11, 1999 on the application, and the prior art references cited therein.

4. I have noted the Examiner's position in the Final Office Action that the claims are broadly drawn to any type of expression vectors comprising a hepatocyte growth factor (HGF) gene and any route of administration of the expression vector to treat a patient. Also, I have noted the Examiner's position that the claimed liposome comprising an expression vector containing the HGF gene and fused to Sendai virus and the treatment method using the liposome would be obvious over Thierry et al. or Isner each in view of Morishita et al.

5. However, as discussed in detail below, I believe that the specification provides sufficient enablement disclosure for direct administration of any type of expression vectors containing the HGF gene to a target site or injured tissue, and for intramuscular administration of any type of the expression vectors. Further, I believe that the direct administration or intramuscular administration of the expression vector for treating a subject in need of HGF is unobvious over any of the prior art references.

The Present Invention

6. I have been involved with research on the feasibility of a gene therapy using a HGF gene for many years, and have found that such a gene therapy can be applied to the treatment of various diseases. Recently, the gene therapy using the HGF gene has reached the stage of its application to clinical trial. Thus, according to the present invention, I have gained the assurance that the HGF gene would enable the gene therapy, and such my assurance is now going to be confirmed in the clinical trial stage. In Japan, many major news papers have interested in and recently

announced our clinical trial plan for the gene therapy using the HGF gene. The copies of the Japanese News Papers are attached hereto together with the English translations
thereof.

7. The present invention has, for the first time, ascertained the feasibility of the gene therapy using the HGF gene. In other words, it is not too much to say that my basic concept for the potential gene therapy using the HGF gene has been realized and completed according to the present invention.

8. At the time when the Japanese applications, based on which the present application claims the priority under the Paris Convention, were filed in Japan, it had already been confirmed and well acknowledged in the art that the HGF protein is effective in the treatment of various diseases. However, nothing whatsoever had been made clear for the feasibility of the gene therapy using the HGF gene. Indeed, it had been entirely unclear whether the HGF gene after transfected would be expressed to produce the HGF protein, and whether the HGF gene would be effective as a gene therapy. The HGF protein is composed of 728 amino acids

and thus has a high molecular weight of about 100 kDa, and has a complicated structure consisting of α-subunit and β-subunit. Further, the HGF protein can exhibits its activity only after the single chain HGF protein expressed has been cleaved to the double chain protein. From these unique characteristics of the HGF protein, it has been entirely unpredicted whether the HGF gene after transfected would be correctly expressed to produce the single chain protein followed by the processing to the active double chain protein to effectively exhibit its pharmacological activity.

9. I and my co-researchers have first investigated whether the HGF gene could function similarly to the HGF protein after transfected to vascular endotherial cells and vascular smooth muscle cells. In fact, as demonstrated by the <u>in vitro</u> tests at Test Examples 2 - 7 of the present specification, we have made clear that the HGF gene was correctly expressed after transfected to the cells and could function similarly to the HGF protein to stimulate the growth of vascular endotherial cells without replication of vascular smooth muscle cells. Furthermore, we have revealed that the HGF gene could more significantly stimulate the growth of vascular endotherial cells than

the HGF protein, as demonstrated at Test Example 2 of the specification.

10. Then, we have conducted in vivo tests to investigate the effect of the HGF gene. More specifically, as demonstrated at Test Example 8 of the specification, we have made clear that, when the HGF gene was administered to rat at the heart muscle, the gene could expressed to produce the HGF protein effectively exhibiting the angiogenesis activity, well reflecting the results from the in vitro tests at Test Examples 2 - 7 of the specification. As stated in the Amendment filed on June 30, 1999 in response to the Office Action dated December 30, 1998 on the above-identified application, the cells and animal models used in Test Examples 2 - 8 are well recognized in the art as correlating with a human diseases. Accordingly, the results from Test Examples 2 - 8 have revealed that the HGF gene is applicable to the treatment of arterial disease.

11. In the meantime, it has been already made clear and confirmed that the HGF protein is effective in the treatment of various diseases, as described in detail in the specification at pages 1 to 2. It is well

known in the art that the functions of the HGF protein is mediated by a HGF receptor, c-Met. That is, the HGF protein binds to the c-Met receptor to exhibit its biological activities such as cell growth stimulation on vasculars and tissues in various organs including liver, kidney, epithelium, brain nerve, lung and cartilage. Thus, the biological various activities of the HGF protein on various vasculars and tissues are based on the common mechanism via the c-Met receptor.

12. As stated hereinabove, the results from Test Examples 2 - 8 of the specification have, for the first time, revealed that the HGF gene is applicable to the treatment of arterial disease. Thus, from the finding that the HGF gene is applicable to the treatment of at least one disease such as arterial disease, we could have then hightly expected that the HGF gene might be similarly effective in the treatment of other diseases, namely, the HGF gene might effectively function against other diseases based on the common mechanism via the c-Met receptor and therefore might be effective in the treatment of the other diseases for which the HGF protein is effective.

13. Under this expectation, as shown in Test Example 9 of the specification, we have investigated the biological action of the HGF gene on cartilage cells, which is considered to be definitely different from the angiogenesis activity of the HGF gene. In fact, we have elucidated the cartilage repair effect of the HGF gene when administered to a rat model with injured joint cartilage, and confirmed that the administration of the HGF gene has expectedly resulted in the significant repaire of the injured joint cartilage.

14. From the results of Test Examples 2 - 9 of the specification, I have gained the assurance that the HGF gene would be effectively applicable to the treatment of almost of the diseases for which the HGF protein is effective. In this regard, the specification states at page 9, lines 8 - 10 as follows:

-- The "pharmaceutical composition" used in the present invention indicates a medicament for the treatment or prevention of human diseases, which is attributed to the pharmacological activities of HGF. For example, exemplified are medicaments for the treatment or prevention of the diseases given hereinabove. According to the present

invention, the HGF gene is introduced into cells wherein HGF is expressed in those cells to exhibit the pharmacological actions. Thus, the medicament of the present invention is effectively applicable to the diseases for which HGF itself is effective. --

15. After the present application was effectively filed in the U.S.A., some articles reporting experimental data confirming the effects of the gene therapy using the HGF gene according to the present invention have been published, as listed below:

① Ueda et al., Supplement to Circulation, Vol.96, No.8, October 21, 1997, Abstract from the 70th Scientific Session, I - 619.

This article reports that the gene therapy using the HGF gene was effective for ischemia-reperfusion injury in the heart.

② Ueki et al., Nature Medicine, Vol.5, No.2, February 1999, 226 - 230.

This article reports that the gene therapy using the HGF gene was effective for liver cirrhosis.

③ Yaegashi et al., The Welfare Ministry Specific Diseases, Respiratory Diseases Research Group, Pervaded Lung Disease Sub-group, 1997 Research Report, 51 - 53.

This article reports that the gene therapy using the HGF gene was effective for injured lung.

④ Ueda et al., Ann. Thoracic Surgeons, 1999; 67: 1726 - 1731.

This article reports that the gene therapy using the HGF gene was effective for ischemia-reperfusion injury in the heart.

16. Further, the inventors of the present invention have authored two articles unpublished but in press, as listed below:

⑤ Taniyama et al, Therapeutic Angiogenesis Induced by Human Hepatocyte Growth Factor Gene in Rat and Rabbit Hind Limb Ischemia Model.

This article reports that the gene therapy using the HGF gene was effective for hind limb ischemia.

⑥ Aoki et al., Angiogenesis Induced by Hepatocyte Growth Factor in Non-infarcted Myocardium and Infarcted Myocardium.

This article reports that the gene therapy using the HGF gene was effective for infarcted myocardium on the same procedure as that used in Test Example 8 of the present specification.

17. All the copies of Articles ① - ⑥ as listed above are attached hereto, and the copies of Articles ① and ② were also attached to the Amendment filed on June 30, 1999 in the above-identified application.

Rejection under 35 U.S.C. § 112, First Paragraph in the Final Office Action

18. I have noted that the Examiner has stated in the Final Office Action at page 3, line 17 to page 4, line 2 as follows:

-- This evidence is deemed to provide sufficient support for the enablement of a pharmaceutical composition comprising a liposome fused to Sendai virus and comprising a plasmid expression vector containing the HGF gene and a method of treating patients with the pharmaceutical composition by direct administration to the target site.--

Thus, the Examiner has admitted that the evidences, Articles ① and ② as stated hereinabove, sufficiently support the pharmaceutical composition for use as a method of treatment when directly administered to the target site. With regard to the direct administration to the target site, I would like to point out that Articles ④, ⑤ and ⑥, in addition to Articles ① and ②, further sufficiently support the direct administration to the target site.

19. The direct administration includes the embodiment wherein the pharmaceutical composition containing the HGF gene is directly administered to an injured tissue. Such an embodiment is specifically described in Test Examples 8 and 9 of the specification.

Further, Articles ⑤ and ⑥ support the direct administration of the HGF gene to an injured tissue.

20. In the meantime, according to the present invention, the pharmaceutical composition containing the HGF gene may be preferably administered intramuscularly to the subject in need of the HGF protein. In this regard, Articles ②, ⑤ and ⑥ report that, when the HGF gene was intramuscularly administered to each of the animal models with liver cirrhosis, hind limb ischemia and infarcted myocardium, the HGF gene was effective for those diseases on those different organs. These evidences fully support the effective intramuscular administration of the HGF gene, together with the statements "The medicament may be administered ····· intramuscularly" in the specification at page 13, the last line to page 14, line 2 as well as Test Example 8 of the specification wherein the HGF gene was administered to the heart muscle of the rat.

21. In view of the foregoing, I believe that the above-identified application should enable a person skilled in the art to practice the direct administration of the HGF gene to the target site or injured tissue

and the intramuscular administration of the HGF gene as well.

22. I have noted that, in the Final Office Action dated August 11, 1999, the Examiner has accepted only the Sendai virus-fused liposome (HVJ-liposome) encapsulating the plasmid vector containing the HGF gene as the dosage form in the present invention. However, I entirely disagree with the Examiner's position, and the Examiner's position should be unfounded for the reasons set forth below.

23. Since the Test Examples of the specification have demonstrated and confirmed that the gene therapy using the HGF gene is effective for various diseases, we could have highly expected, in view of the extremely potent activity of the HGF protein per se as a cell growth stimulation factor, that any dosage forms of the HGF gene according to a viral expression vector method, a naked-DNA method using an expression plasmid containig the HGF gene, and the like would effective as the gene therapy similarly to the HVJ-liposome method used at the Test Examples of the specification. In this regard, the specification states at page 9, lines 5 - 8 and page 12, lines 18 - 24, respectively, as follows:

-- ····· the HGF gene may be used in the form of a viral vector having the HGF gene as described hereinafter, or in the form of an appropriate expression vector having the HGF gene. --

-- For introduction of the HGF gene into cells, conventional methods are employed, which are roughly classified into introduction via viral vectors and other strategies. Both methods are available for the preparation of the medicament of the present invention. --

24. Indeed, the above statements in the specification are supported by the experimental data in Articles ③ and ⑤ as listed hereinabove. More specifically, Articles ③ reports that the transfection of the HGF gene to the animal model with injured lung according to Adenoviral vector containing the HGF gene was effective as the gene therapy using the HGF gene. Also, Article ⑤ reports that the intramuscular injection of "naked" human HGF plasmid into rat hindlimb ischemia model resulted in a significant increase in blood flow as stated at page 3 , lines 8 - 10. The naked-DNA method using the "naked" human HGF plasmid is a simple and

convenient method wherein a non-viral expression vector containing the HGF gene is directly transfected to the subject in need of the HGF protein without the use of viral particles, liposomes and the like. Therefore, according to even the naked-DNA method, the gene therapy using the HGF gene is also effective in the present invention.

25. As is clear from the above statements, the use of any dosage forms of the HGF gene is effective in the present invention, which I believe is sufficiently supported by the descriptions in the specification of the above-identified application and has been confirmed by the Articles published after the effective filing date of the present application.

26. In the meantime, I have noted that, in the Final Office Action, the Examiner has pointed out based on the teachings of Kohn et al. that "the actual gene therapy treatment was a disappointment, most likely due to the inefficient gene delivery system." As pointed out by the Examiner, the gene therapy technique might not yet, in general, been established in the art. However, I believe that this general knowledge in the art should not be applied to the gene therapy using the

HGF gene. As discussed in detail hereinabove, it has been well acknowldged in the art that the HGF protein per se functions via the c-Met receptor to be effective for various diseases. Further, the Japanese News Papers attached hereto has announced that the HGF protein have attracted public attention due to the extremely potent activity for regenerating liver even if cut off the half of the liver. Also, Articile ⑦, Francesco Galimi et al., STEM CELLS, 1993; 11: 22 - 30, of which copy is attached hereto, states at page 22, the right column, lines 11 - 15, as follows:

> -- HGF is considered to be the major mediator of liver regeneration in vivo; it is a powerful mitogen for several cell types, including hepatocytes, kindney tubular epithelium, keratinocytes, endothelial cells and melanocytes. --

Thus, the HGF protein is well known in the art to be an extremely potent cell growth stimulation factor. In view of the unique characteristic property of the HGF protein, since the Test Examples of the specification have demonstrated that the HGF gene is effective as the gene therapy, I have had the assurance that any dosage forms

of the HGF gene would be used as the gene therapy. Indeed, my this assurance has been confirmed by the experimental data in the Articles published after the effective filing date of the present application.

Rejection under 35 U.S.C. § 103 (a) in the Final Office Action

27. I have noted that, in the Office Action, the Examiner has considered that the present invention using the HVJ-liposome (Sendai virus-fused liposome) is obvious over Thierry et al. or Isner each in view of Morishita te al. However, I believe that the present invention using any dosage forms in addition to the HVJ-liposome should have inventive step over any cited prior art references, for the reasons as set forth in detail below.

(1) Treatment method using HVJ-liposome containing HGF gene

28. Thierry et al. does not describe a HVJ-liposome but merely teaches the method for encapsulating high molecular weight nucleic acids in liposomes. Noticeably, Thierry et al. describes nothing

whatsoever of the specific treatment of diseases. Morishita et al. does not describe anything of the HGF gene and further provide nothing whatsoever of the specific treatment of diseases, although Morishita et al. teaches a HVJ-liposome.

29. Isner teaches the treatment of arterial diseases, as pointed out by the Examiner in the Final Office Action. However, Isner does not specifically describe the effect of HGF gene, although Isner provides the pharmacological data for a VEGF gene. Even though the VEGF gene was effective as taught in Isner, a person skilled in the art could not have predicted whether the HGF gene might be similarly effective, for the reasons as given below.

30. At the outset, I would like to emphasize that the HGF protein is definitely different as the substance from the VEGF protein. More specifically, the HGF protein has a molecular weight of about 100 kDa which is larger by twice than that of the VEGF protein. Further, the HGF protein is initially produced in vivo as a single chain protein, and then cleaved to the double chain protein to exhibit its biological activity. In this regard, the HGF protein is entirely contrasted

to the VEGF protein in the mechanism for exerting the biological activity. Therefore, even if the VEGF gene was effectively transfected, it could not have been predicted whether the HGF gene after transfected would be correctly expressed and processed to the double chain protein for exerting its biological activity.

Furthermore, the receptor of the HGF protein is c-Met, which is different from the receptor of the VEGF protein, resulting in the different activity on vascular cells. Indeed, the VEGF protein has inclusively three activities, angiogenesis activity, vascular permeation activity and vasodepressor activity, which are important to the biological activity of the VEGF protein. In contrast, the HGF protein has substantially only angiogenesis activity, and has no vascular permeation activity. Thus, in view of these differences between the HGF and VEGF proteins, a person skilled in the art could not have predicted whether the HGF gene might be effective as the gene therapy. In this regard, Isner does not teach anything. Isner states from the column 3, line 40, as follows:

> -- "Such proteins include, for example, acidic and basic fibroblast growth factors (aFGF and bFGF), vascular endotherial growth factor

(VEGF), ····· hepatocyte growth factor

(HGF) ···· --

Thus, Isner exemplifies all the possible angiogenesis factors, but the bFGF gene encoding the exemplified bFGF protein would be suspected to be ineffective as a gene therapy due to no signal sequence, although the bFGF protein has a potent angiogenesis activity. Under these circumstances, even though a protein was known to have a potent angiogenesis activity, a person skilled in the art could not have predicted whether the protein gene might be effective as a gene therapy, before conducting experiments to confirm the effectiveness as the gene therapy. Furthermore, Isner describes the different procedure wherein a catheter having hydrophilic polymers containing the VEGF gene attached to the end thereof was inserted to arterial vessel whereby the VEGF protein expressed was acted on the arterial vessel. Thus, the procedure of Isner was quite unique and complicated and therefore entirely different from the administration method of the present invention wherein the HGF gene is directly administered to the injured tissue or intramuscularly administered.

31. Accordingly, the views of the Examiner should be unjustified that "applicants' showing that HGF liposomes enable efficient transfection and expression of the HGF gene in arterial cells is exactly what one of ordinary skill in the art would expect following a reading of the cited references." In view of the foregoing discussions, I believe that the treatment method using the HVJ-liposome containing the HGF gene according the present invention is not obvious over Thierry et al., Isner and Morishita et al., singly or in combination therewith. Furthermore, I also believe that the treatment method using any dosage forms of the HGF gene is not obvious over Thierry et al., Isner and Morishita et al., singly or in combination therewith.

(2) <u>Pharmaceutical composition comprising HVJ-liposome containing HGF gene</u>

32. The pharmaceutical composition comprising HVJ-liposome containing the HGF gene according to the present invention indicates a pharmaceutical composition actually exhibiting a therapeutic effect for diseases. On the other hand, Thierry et al. describes nothing of a HVJ-liposome, although Thierry et al. teaches a liposome. Thierry et al. teaches no more than the method for

encapsulating high molecular weight nucleic acids in liposomes. Thierry et al. describes neither the in vivo effect nor the therapeutical effect of the liposomes encapsulating the nucleic acids.

33. Isner et al. does not teach anything of a HVJ-liposome actually exhibiting the pharmacological effect. Such a liposome would not be obvious to a person skilled in the art from the teaching of Isner et al. Morishita et al. does not teach anything whatsoever of the HGF gene, although Morisihita et al. describes a pharmaceutical composition comprising a HVJ-liposome. Morishita et al. merely describes a gene encoding ACE (angiotensin converting enzyme) irrelevant to the HGF gene. Furthermore, Morishita et al. teaches no more than that the ACE gene was transfected ex vivo to the cultured cells of vessel enucleated from rat and that the expression of the ACE gene was confirmed by immunostaining the cells. Morishita et al. does not teach anything of whether the ACE enzyme expressed in vivo would actually exhibit its enzymatic activity and therapeutical effect.

34. On the other hand, the HGF protein definitely differs from ACE enzyme in that the HGF

protein is a physiologically active protein having a complicated three-dimensional structure and requires various biological steps including the processing of the single chain protein to the double chain protein for exerting its biological functions. For the HGF gene encoding such a complicated protein, we have, for the first time, confirmed the therapeutical effect of the HGF gene when transfected to cells. Even though Morishita et al. teaches the transfection of the ACE gene into the cultured vascular cells, a person skilled in the art could not have easily arrived the pharmaceutical composition comprising the HVJ-liposome containing the HGF gene actually exhibiting the therapeutical effect, as achieved by the present invention.

35. In view of the foregoing discussions, I believe that the pharmaceutical composition comprising the HVJ-liposome containing the HGF gene according to the present invention is not obvious over any of the cited references singly or in combination therewith.

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and

belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 20th day of January, 2000.

Ryuichi MORISHITA